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Recent Advances in the Care of the Patient with Malignant Melanoma

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Objective

The authors review the recent advances in the surgical care, staging, and adjuvant treatment of the patient with melanoma.

Summary Background Data

Melanoma care has not changed significantly in the last 20 years, and the controversy of elective lymph node dissections in this disease continues to be discussed. Two advances in the care of the patient with melanoma have occurred in the last 3 years to make this an exciting time for clinicians and to offer more hope for the patients with this disease. The concept of the sentinel lymph node (SLN), defined by Morton as the first node in the lymphatic basin that drains the primary melanoma, has been documented to contain the first site of metastatic disease. This technology can be used to stage nodally the melanoma patient, identifying the subgroup of patients (stage III) who have a 5-year survival rate less than 50%. Members of this group are candidates for effective adjuvant therapies.

Methods

A review of the surgical techniques of melanoma care, including recently reported new studies of elective node dissection (ELND) and SLN biopsy in patients with melanoma was performed. In addition, the Eastern Cooperative Oncology Group (ECOG) 1684 trial, which was the basis for the Food and Drug Administration approval of adjuvant interferon-alpha–2b (IFN-α–2b) is discussed.

Results

The Intergroup Melanoma Trial has reported a survival benefit for performing ELND in patients with melanoma and tumor thickness between 1 and 2 mm or in patients that are younger than 60 years of age. With six reports in the literature that show there is an order to melanoma nodal metastases and that the SLN histology is reflective of the histology of the remainder of the nodal basin, the more conservative SLN biopsy can be performed to adequately stage nodally the patient with melanoma. Patients with nodal metastases who are rendered free of disease with surgical resection have the most to benefit from adjuvant IF-α–2b. If one considers only the lymph node-positive group of patients, the survival benefit associate with adjuvant IFN is significant ($p = 0.008$).

Conclusions

New standards of care for the melanoma patient have been established. Patients at high risk for recurrence have been shown to experience a survival benefit with adjuvant IFN-α-2b. With these data, the argument can be made that all patients with melanoma greater than 1 mm should have a nodal staging procedure. Selective lymphadenectomy with SLN biopsy is the least morbid procedure that can be used to obtain this information. If surgeons do not have the nuclear medicine or pathology support to perform lymphatic mapping, then the guidelines of the Intergroup Melanoma Study should be used to apply ELND in a selective fashion. In this way, patients are identified with micrometastatic disease early in their clinical course and can be offered the survival benefit of adjuvant therapy.

Cutaneous melanoma is becoming an ever more common disease. In 1995, melanoma developed in an estimated 34,100 individuals, and 7200 died of the disease in the United States. In 1996, an estimated 38,300 will receive a diagnosis of the disease, an astounding 12% increase in incidence. This figure has increased during the last decade at a rate faster than any other cancer. Each day, 12 women and 7 men die of melanoma, and $1.25 billion is spent each year on the care of the melanoma patient.[1]

At the same time, a number of scientific studies have matured to the point that definitive reports have appeared in the literature that have changed the standard of care for the patient with malignant melanoma. These would include studies involving the surgical care of the disease that define the role of elective lymph node dissection (ELND) and the emerging technology of lymphatic mapping in identifying the sentinel lymph node (SLN), defined as the first node in the lymphatic basin into which the primary site drains. Surgical care of the patient with melanoma promises to become more conservative as only those patients with a positive SLN are exposed to the expense and morbidity of a complete node dissection. Finally, the first report in the literature and subsequent Food and Drug Administration approval of an effective adjuvant therapy for high risk for recurrence melanoma (interferon-alpha-2b [IFN-α-2b], Intron A, Schering-Plough, Kenilwood, NJ) has changed the medical standards for this disease. Interferon α-2b provides a survival benefit for these patients that previously were forced to live with an unacceptable rate of recurrence and death from this disease. New, more sensitive molecular biology techniques used to identify micrometastatic disease promise to provide a more accurate staging for the patient with melanoma, so that patients are not denied adjuvant therapy because of missed micrometastases. In this way the adjuvant therapy can be applied in a selective fashion, so that only those patients with the most to benefit are exposed to the toxicities. "The times are a-changing" for the better for patients with the diagnosis of melanoma.

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MOLECULAR BIOLOGY TECHNIQUES FOR THE STAGING OF MELANOMA

For most solid tumors, including melanoma, the most powerful predictor of survival is the presence or absence of lymph node metastases. Once lymph node metastases occur, variables based on the primary tumor add little to the prognostic model. The presence of lymph node metastases in patients with melanoma decreases the 5-year survival rate approximately 40% compared with those who have no evidence of nodal metastases. Much time, effort, and expense are placed on identifying prognostic factors based on the primary tumor, whereas not enough emphasis has been given to identify patients who have micrometastatic disease in their nodal basins. For instance, there currently are 26 prognostic factors (Table 1) for melanoma based on variables from the primary tumor. However, in multiple regression analyses performed on a number of populations, lymph node status remains the most powerful factor for predicting recurrence and survival. Primary tumor variables, such as Breslow thickness, ulceration, primary site, and gender, may add to the prognostic model, but only after nodal status is considered. It also is clear that routine histologic examination of the regional lymph nodes, an examination that typically involves making one to two sections of the central area of the node and staining with a standard stain, examines less than 1% of the submitted material and often will miss micrometastatic disease. The sensitivity of the routine histologic examination is identifying 1 abnormal melanoma cell in a background of 10^4 normal lymphocytes. If serial sectioning and immunohistochemical staining is added, the yield of positive dissections may double, and the sensitivity becomes the identification of 1 abnormal melanoma cell in a background of 10^5 normal lymphocytes. Serial sectioning and immunohistochemical staining techniques have been available for years, yet they have not been incorporated into the routine practice of

Table 1. PROGNOSTIC FACTORS FOR MELANOMA BASED ON THE PRIMARY TUMOR*

Tumor thickness
Ulceration
Clark level
Histologic type
Cell type
Primary site
Regression
Mitosis
Lymphocytic infiltration expression
Vertical maturation grade expression
Blood vessel invasion
Lymphatic space invasion
Ploidy
S phase
DR-1 expression
DNA index
Heat shock protein expression
HLA-DR staining
p53 mutations
Cell adhesion molecular expression
Proteases expression
Migration-associated molecule
Angiogenesis-related factor
Oncogene expression
Estrogen receptor expression
Cytokine, growth factor expression

HLA = human leukocyte antigen.
* From Reintgen DS, et al. Accurate nodal staging of malignant melanoma: cancer control. J Moffitt Cancer Center 1995; 2:405–414.

the pathologist for examining nodes because of the time and expense involved. In addition, the rate limiting step continues to be the number of sections stained and examined, which can be an expensive, time-consuming proposition.

New technology that enables the surgeon to map the cutaneous lymphatic flow from the primary tumor and identify the SLN in the regional basin could contribute to better nodal staging of the melanoma patient. This procedure, as initially proposed and proven by Morton et al.,[2,3] has shown that the SLN is the first site of metastatic disease and that if the SLN is negative for disease, then the remainder of the lymph nodes in the basin also should be negative.[4,5] Selective lymphadenectomy also allows for the detailed examination of the SLN because it is an examination of one or two nodes. This advance allows the pathologist to serial-section the node and use immunohistochemistry to look for micrometastatic disease. Nevertheless, 25% of the histologic node negative stage I and stage II melanoma patients will have recurrence and die of their disease within 5 years of diagnosis, suggesting that some of these patients have occult nodal micrometastases after routine examination or suffer from hematogenous metastases. If prognosis and treatment decisions are dictated by the presence or absence of nodal metastases, a more sensitive method is needed to accurately identify the presence or absence of metastatic disease in the node.

Investigators at the Moffitt Cancer Center (MCC), University of South Florida, Tampa, initially proposed a cell culture technique,[6] in which the regional nodes were bisected and half the node was sent to pathology and half the node was placed into tissue culture. Thirty-one percent of the histologic node-negative population were upstaged to stage III with the cell culture technique. Correlation was found in those patients who were histologically node negative, but whose culture was node positive, with an increase recurrence rate compared with those patients with melanoma whose nodes were negative with both assays.[7]

A study[8] was initiated to develop a more reproducible, highly sensitive method to detect micrometastases by examining lymph nodes for the presence of tyrosinase messenger RNA, a material found almost exclusively in melanocytes. The hypothesis was that if messenger RNA for tyrosinase was found in the lymph node preparation, then that is good evidence that metastatic melanoma cells are present.

The assay was created and refined using the combination of reverse transcription polymerase chain reaction (RT-PCR) and double round PCR. Lymph nodes from 29 patients were analyzed by standard pathologic staining and RT-PCR. Eleven of 29 lymph node samples (38%) from 29 patients with intermediate-thickness melanoma were pathologically positive. Nineteen of the 29 lymph node preparations (66%) were RT-PCR positive, and these included all of the pathologically positive samples. Additional experiments demonstrate that the RT-PCR technique can identify 1 melanoma cell in 1 million normal lymphocytes, which is two orders of magnitude greater in sensitivity than routine histology. From this study, it was concluded that the RT-PCR method was an extremely sensitive, specific, reproducible, and efficient technique for the identification of micrometastases in patients with melanoma. Furthermore, this technique is widely applicable because thermocyclers have become more widely available.

Table 2 shows the initial clinical correlation of the PCR assay.[9] Patients whose SLNs were histologic positive and PCR positive had a recurrence rate of 42% at 3 years, whereas patients whose SLNs were negative with both assays did well, with a recurrence rate of 6.6% at 3 years. The interesting group was composed of those patients whose SLNs are histologically negative but PCR positive. These patients are upstaged with the PCR assay and have an intermediate prognosis, with 22% recurring at 3 years. In addition, RT-PCR positivity of the regional nodes or

Table 2. CLINICAL CORRELATION OF NODAL STATUS WITH REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)*

Nodal Status Systemic	No. of Patients	Recurrences	Local	Regional
Histology + RT-PCR +	14	6 (42%)	2	4
Histology − RT-PCR +	27	6 (22%)	3	3
Histology − RT-PCR −	33	2 (6.6%)	1	1

* From Reintgen DS, Albertini J, Berman C, et al. Accurate nodal staging of malignant melanoma: cancer control. J Moffitt Cancer Center 1995; 2:405–414.

SLNs correlates with tumor thickness at diagnosis, a prognostic factor that is related linearly to survival of the patient with malignant melanoma (Fig. 1). Patients with relatively thin melanomas (range, 0.76–1.5 mm) have an RT-PCR node-positive rate of 33%, whereas 80% of the individuals with thick melanomas have the presence of tyrosinase messenger RNA in the regional node.

Reverse transcription-PCR assays for tyrosinase gene products in the peripheral blood may be one of the more promising serum markers for melanoma staging and for predicting recurrence, prognosis, and response to therapy.[10] Brossart and coworkers[11] have shown that with PCR, messenger RNA for the tyrosinase gene can be identified in the peripheral circulation of melanoma patients and the rate of positivity correlates with stage of disease.

Other investigators also have used molecular biology techniques to improve the staging of the melanoma patient. Dale and colleagues[12] used an RT-PCR technique to detect occult circulating tumor cells in the blood of melanoma patients. Four different markers were used to improve the sensitivity and specificity of the occult metastases assay. Preliminary studies of this multiple marker RT-PCR assay, which used the four melanoma markers tyrosinase, MAGE-3, Muc-18, and p97, showed the presence of all four markers in ten melanoma cell lines, with none detected in the blood of 14 healthy volunteers. The assay was used to detect circulating tumor cells in 74 melanoma patients of various clinical stage. The pattern of marker detection was as follows: tyrosinase (59%), MAGE-3 (9%), Muc-18 (66%), and p97 (65%). For all American Joint Committee on Cancer stages, the ability to detect circulating tumor cells was significantly higher ($p = 0.025$) in the 53 patients alive with disease than the 21 disease-free patients. For all patients alive with disease, as the clinical stage increased from I to IV, there was a corresponding increase in the ability of the assays to detect circulating melanoma cells. The detection of circulating occult tumor cells could provide a tumor marker for the early detection of metastatic or recurrent disease or evaluate the response to specific therapeutic modalities.

ELECTIVE LYMPH NODE DISSECTION

The issue of ELND probably is one of the most important current controversies in the management of patients with melanoma. Recently reported results of the Intergroup Melanoma Surgical Trial have shown significant improvement in subgroups of patients based on age and thickness. Historically, two randomized prospective studies involving only extremity melanomas did not demonstrate any survival advantage for ELND,[13,14] whereas three nonrandomized studies involving melanomas from all anatomic sites showed a statistically significant improvement in survival for the subgroup of intermediate thickness melanomas (range, 1–4 mm).[15–17] Although there is unanimous opinion that all melanoma patients do not need ELND, there still is a continuing debate that centers around two issues: 1) is it possible to identify accurately a subgroup of melanoma patients at high risk for microscopic regional nodal metastases and a low enough risk of occult systemic metastases to justify a regional node procedure, and 2) what is the optimal timing of dissection (immediate *vs.* delayed) if such a high-risk group can be identified?

Selection of Patients

Prognostic Factors

Tumor thickness provides a quantitative estimate of the risk for occult metastatic melanoma at regional and distant

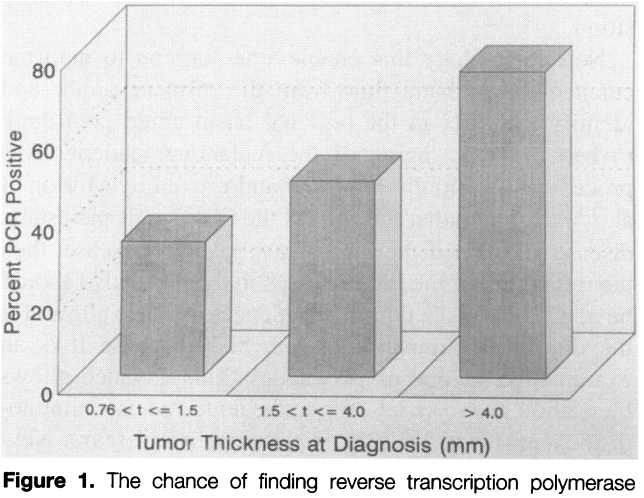

Figure 1. The chance of finding reverse transcription polymerase chain reaction-positve material in the sentinel node increases with increasing tumor thickness at diagnosis. These data give indirect evidence of clinical correlation.

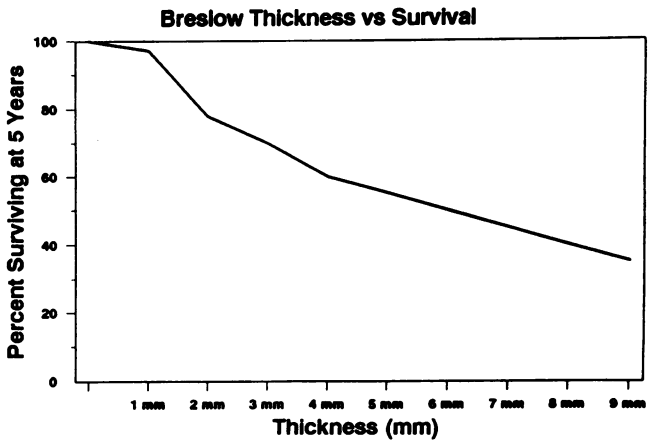

Figure 2. There is a linear relationship between increasing melanoma tumor thickness at diagnosis and decreasing survival.

sites and is the most accurate predictor of survival (Fig. 2). In fact, melanoma thickness is the most important—but not the only—guide for selecting patients who might benefit from ELND.[18] The major advantage of using tumor thickness for these surgical decisions is that it can provide a quantitative estimate of the risk for occult metastatic melanoma in both regional and distant sites.[19] Thin melanomas (<1 mm) are associated with localized disease and a 98% or greater cure rate. Elective lymph node dissection would provide no therapeutic benefit in such patients. Patients with intermediate thickness melanomas (range, 1–4 mm) have an increased risk (up to 60%) of harboring occult regional metastases, but have a relatively low risk (<20%) of distant metastases. Therefore, patients with these lesions might benefit from ELND.[20] Patients with thick melanomas (>4 mm) are at high risk for regional nodal micrometastases (>60%) and also have a high risk (>70%) of occult distant disease at the time of initial presentation.[21] These patients do poorly as a group because the distant metastases in most instances negate any benefit of surgically excising the regional lymph nodes. The treatment goal of removing these nodes is palliative, and the operation might be deferred until nodal metastases become clinically evident. Some surgeons prefer to perform ELND as expectant palliation in patients with thick melanomas to avoid the probability (approximately 40%) of a second operation for lymph node metastases.[22] Elective lymph node dissection also might be justified as a staging procedure to document the pathologic status of the lymph nodes in patients with thick melanomas before entry into clinical trials involving systemic adjuvant chemotherapy or immunotherapy.

Tumor thickness should not be the sole criterion for making surgical treatment decisions. Other factors, such as the presence or absence of tumor ulceration,[23] the patient's gender[24] and age,[25] the anatomic location of the melanoma, and the operative risk, all should be considered when making the decision to perform ELND on any individual patient. For instance, for melanomas less than 1 mm thick, a nodal procedure may be considered if the primary lesion is found on an axial location of males, is a Clark Level IV or greater melanoma, is ulcerated, or shows regression that suggests that at one time the lesion was thicker.[26]

Identifying the Regional Lymph Node Basins at Risk for Metastases

Because melanomas located on the trunk and on the head and neck area have unpredictable lymphatic drainage, it is difficult to decide which nodal basin is at risk for metastatic disease. In many patients, this problem has been overcome by performing a radiocolloid cutaneous scan that can accurately define the location of nodes that are the primary drainage site for a melanoma located anywhere on the body. Lymphoscintigraphy, a nuclear medicine test that involves a radiocolloid injection around the primary site and a scan of the regional basins, has been used to redefine cutaneous lymphatic flow that has proven to be different than what is described in classical anatomic descriptions.[27]

Lymphoscintigraphy, using technetium antimony trisulfide, has been performed in the Sydney Melanoma Unit (SMU)[28] to show the great variability in lymph node drainage patterns with the identification of several new lymphatic drainage pathways. Twenty-six percent of melanomas on the back drain into an in-transit node located in the triangular intramuscular space; 20% of the periumbilical melanomas show an initial drainage into an internal mammary node. Direct drainage has been noted from the back into para-aortic and mediastinal lymph nodes, and at least one forearm melanoma showed direct drainage to a supraclavicular node. A series from MCC[29] has reported on more than 400 studies of patients with clinical stage I and II disease. With a mean follow-up of 5 years, there has been no metastases in regional nodal basins not visualized by lymphoscintigraphy. Lymphoscintigraphy is an extremely accurate method of identifying all nodal basins at risk for metastatic disease, and unless performed preoperatively, the ELND or sentinel node biopsy may be misdirected in up to 50% of the cases. The advantage of lymphoscintigraphy over classic anatomic guidelines is the scan's reflection of functional and not just structural anatomy. The results from the MCC and the Sydney Melanoma Unit show that lymphoscintigraphy can be used as a road map so that the surgeon does not perform too little or too aggressive surgery. The results of these series also cast into doubt the conclusions of studies of ELND, in which preoperative lymphoscintigraphy was not used as part of the protocol. Of the recently completed prospective randomized trials evaluating the efficacy of ELND,

only the Intergroup Melanoma Study required lymphoscintigraphy before ELND.

Results of Randomized Clinical Trials Involving Elective Lymph Node Dissection

Four prospective trials to evaluate ELND in the treatment of stage I and stage II melanoma have been performed: two international cooperative studies conducted by the World Health Organization (WHO) Melanoma Group,[13,30] a study by surgeons at the Mayo Clinic,[14] and the Intergroup Melanoma Trial headed by Balch.[31] These studies clearly demonstrated that all patients did not benefit from ELND. However, the question remained whether any subgroup of patients might benefit. The first three studies included melanoma patients of all thicknesses, whereas the Intergroup Surgical Trial specifically addressed the potential benefit of ELND in the subgroup of intermediate-thickness melanomas.

The initial WHO Melanoma Group study involved 553 patients with stage I and II primary melanoma in the distal two thirds of the limbs. Of these patients, 286 (52%) were randomized to receive wide excision of the primary melanoma as initial treatment and node dissection only if regional nodes became clinically detectable; 267 (48%) received wide excision plus ELND. The two groups were matched according to the major prognostic criteria. No differences in survival were noted between the two groups.[13] Because subgroups of patients may have benefitted from ELND, survival was evaluated according to prognostic criteria: gender, invasion levels III and IV, tumor thickness, and ulceration. No significant survival differences were noted in any of these subgroups. However, a separate analysis of the data demonstrated a 22% increase in 10-year survival rate in a small subgroup with intermediate-thickness lesions.[32]

In the second trial from the WHO Melanoma Program (Trial #15) for patients with high-risk trunk melanoma, there also was no difference for the entire population in the study according to primary surgical therapy.[30] Subgroup analysis performed in this study showed that men with melanomas less than 4 mm in thickness who were treated with a wide local excision and ELND had a significant survival advantage compared with the control population, whose primary surgical therapy consisted of a wide local excision alone ($p = 0.03$).[15] Men with trunk melanomas also had a significant increase in survival ($p = 0.04$). In a multivariate regression analysis, gender ($p = 4 \times 10^{-5}$ and tumor thickness ($p = 0.004$) remained significant, whereas ELND lost some of its significance ($p = 0.08$). A major criticism of these two trials includes the fact that preoperative lymphoscintigraphy was not performed. Although WHO Trial #1 included only extremity melanoma in which the cutaneous lymphatic flow is relatively straightforward, in-transit metastases would be missed in 5% of the cases. For the WHO Trial #15, without preoperative lymphoscintigraphy, ELND may have been misdirected in up to 50% of the surgical procedures.[27]

Surgeons at the Mayo Clinic conducted a clinical study from 1972 to 1976 in which 171 patients with stage I melanoma were randomized into one of three treatment groups: 1) 62 patients had their nodes left intact; 2) 55 patients had ELND that was delayed 30 to 60 days after the primary melanoma excision; and 3) 54 patients had elective lymphadenectomy concomitantly with the primary melanoma excision.[14] Patients with lesions of the head and neck and midline trunk were excluded.

In this study, the subgroup of patients who did not have ELND was older, consisted of more men, and had worse prognostic features (*i.e.*, deeper invasion, thicker lesions, and more nodular lesions) than the two subgroups who underwent ELND. The subgroup that received immediate ELND had more sites involving the trunk than the other subgroups. None of these differences was statistically significant, although the subgroup with intact nodes was biased toward an unfavorable prognosis with other factors. Six characteristics were analyzed: initial surgical treatment, age, gender, anatomic site, tumor thickness, and growth pattern. The only factors that were significantly related to survival were tumor thickness ($p < 0.0001$) and growth pattern ($p = 0.02$). When overall survival and disease-free survival of the three surgical treatment groups in the Mayo Clinic study were compared, there were no significant differences.[14] The 5-year survival rate was 85% when the nodes were left intact, 85% when the nodes were removed immediately, and 91% when delayed ELND was performed. Survival and disease-free survival were related significantly to the thickness of the lesion. This trial suffers from the fact that numbers of patients are insufficient to make any meaningful conclusions when so many prognostic variables of the primary tumor, such as tumor thickness, ulceration, primary site, and gender, need to be controlled in the multivariate analysis. Power calculations would suggest that the minimum number of patients needed in a three-arm randomized trial controlling for the five variables with minimal estimated survival differences between the groups would be close to 1000 patients. The trial also did not include preoperative lymphoscintigraphy to identify those patients with in-transit metastases and identify all basins at risk for disease, casting doubt on the conclusions.

Thus, both the Mayo Clinic and the WHO Melanoma Group studies indicated no benefit from routine ELND for patients with stage I and II melanoma involving the extremities and trunk. However, there are still legitimate differences in interpreting the results of the two trials, and these can be resolved only by continuing to perform

randomized clinical trials using current stratification criteria, extending these studies to all anatomic sites, but confining the patient eligibility to intermediate-thickness melanomas.

The recently completed Intergroup Melanoma Trial fulfilled the aforementioned criteria because it was designed to address the efficacy of ELND in subgroups of patients with intermediate-thickness melanomas in a large population of patients stratified for major prognostic factors and included preoperative lymphoscintigraphy to identify all nodal basins at risk for metastatic disease.

In the multi-institutional trial, 742 patients with intermediate-thickness melanomas (range, 1–4 mm) and clinically negative lymph nodes were allocated randomly to receive an ELND or observation of their nodes as their initial management. The patient groups were stratified according to tumor thickness, primary site, and ulceration. The patients have been followed for a median of 8.6 years (range, 3.3–12 years).

Overall survival was not significantly different when the two surgical options were compared for the entire group of patients. However, two defined subsets of patients (by age and by tumor thickness) had a significant increase in overall survival with ELND. Among the 555 patients 60 years of age or younger (75% of the total population), those who had ELND had a significantly improved 5-year actuarial survival rate compared with those who had observation (89% *vs.* 80%, respectively; $p = 0.047$). In a multivariate (Cox regression) analysis for patients 60 years of age or younger, the following features independently predicted outcome: tumor ulceration ($p < 0.001$), regional nodal dissection ($p = 0.03$), trunk primary site ($p = 0.03$), and tumor thickness of 2.1 to 4 mm ($p = 0.03$). In contrast, patients 60 years of age and older who had ELND actually had a lower 5-year survival than those who had observation (74% *vs.* 86%; $p = 0.21$).

Among the 396 patients with a tumor thickness of 1.1 to 2 mm (53% of the total group), those who were assigned randomly to ELND had a significantly better overall 5-year survival rate compared with those who had observation (92% *vs.* 84%; $p = 0.05$). In this subgroup of patients, significant factors affecting outcome in the Cox multivariate analysis were tumor ulceration ($p < 0.001$), regional node dissection ($p = 0.041$), and trunk site ($p = 0.045$). Finally, the cohort of 293 patients with tumor thickness of 1.1 to 2 mm who were 60 years of age or younger (39% of the total group) had a significant improvement in 5-year survival rate with ELND compared with observation (96% *vs.* 84%; $p = 0.007$). This was confirmed by multivariate analysis, in which ulceration ($p = 0.001$) and regional node dissection ($p = 0.003$) were the only significant parameters in this subgroup.[31]

Thus, this prospective surgical trial identified major subgroups of patients with significant improvement in overall survival if their initial management included ELND. This is the first randomized study to prove the value of surgical treatment for clinically occult metastatic melanoma. The results of this trial can be used when making surgical recommendations for patients 60 years of age or younger with intermediate-thickness melanomas, especially those whose tumors are 1.1 to 2 mm thick.

INTRAOPERATIVE LYMPHATIC MAPPING AND SENTINEL NODE BIOPSY

A new procedure has been developed to assess the status of the regional lymph nodes more accurately and decrease the morbidity and expense to the healthcare system of a complete ELND. The technique, termed *intraoperative lymphatic mapping and selective lymphadenectomy,* relies on the concept that regions of the skin have specific patterns of lymphatic drainage not only to the regional lymphatic basin, but also to a specific lymph node (sentinel lymph node, SLN) in the basin. Morton[2,3] initially proposed and proved the technique valid using a vital blue dye method and showed that in animals and initial human trials, the SLN is the first node in the lymphatic basin into which the primary melanoma drains. He showed that the SLN histology reflected the histologic status of the remainder of the nodal basin, so that complete nodal staging could be obtained with an SLN biopsy. If the SLN was negative for metastatic disease, the remainder of the nodes in the basin also should be negative.

These data have been confirmed by four other institutions, including the MCC,[4] M. D. Anderson Cancer Center,[5] the Sidney Melanoma Unit,[33] and the University of Vermont.[34] These studies have demonstrated an orderly progression of melanoma nodal metastases. Most solid tumors are thought to demonstrate a random nodal metastatic pattern. The incidence of "skip" nodal metastases, reported in breast cancer in up to 15% of the cases,[35] precluded the use of sampling procedures of first station nodal basins to achieve accurate pathological staging. The data from these surgical trials demonstrated that the pattern of nodal metastases from cutaneous melanoma is not random. The SLNs in the lymphatic basins can be identified individually and they reflect the presence or absence of melanoma metastases in the remainder of the nodal basin. This information is being used to change the standards for melanoma surgical care so that only those patients with evidence of nodal metastatic disease are subjected to the morbidity and expense of a complete nodal dissection.[36] These findings demonstrate accurate pathologic staging, no decrease in standards of care, and a reduction of morbidity (no lymphedema, early return to work or normal activity) with a less aggressive, rational

surgical approach and lower costs for the healthcare system.[37] Then the status of the SLN can be used as a prognostic factor to identify which patients need the more radical complete node dissection.

With the addition of intraoperative radiolymphoscintigraphy to vital blue dye lymphatic mapping,[38] the SLN localization becomes easier and more widely applicable. In the initial study[38] from MCC comparing the vital blue dye and radiocolloid mapping technique, 450 μCi of filtered technetium-labeled sulfur colloid and the standard isosulfan blue was injected at the site of the primary cutaneous melanoma. A nuclear probe (Neoprobe, Columbus, OH) was used to trace lymphatic channels from the primary site to lymph nodes in the regional lymphatic basin. The SLN in the basin was identified by intense radioactivity, and when the node was excised, the high levels of residual activity in the node and the low background activity in the rest of the basin confirmed that the SLN was removed.

This study consisted of 106 consecutive patients with cutaneous melanoma greater than 0.76 mm at all primary site locations. A total of 200 SLNs and 142 neighboring nonsentinel nodes were harvested from 129 basins in 106 patients. When correlated with the vital blue dye mapping, 70% of the SLNs demonstrated blue dye staining whereas 84% of SLNs were defined as "hot" by radioisotope localization. With the use of both intraoperative mapping techniques, identification of the SLN was possible in 96% of the nodal basins sampled. Micrometastases were identified in SLNs in 15% of the patients by routine histology whereas two patients had micrometastatic disease in "hot" but not blue-stained nodes. This suggest that the radiocolloid localization identifies more SLNs, some of which are clinically important because they contain micrometastatic disease.[38] The addition of the radiocolloid mapping allows the identification of the location of the SLN through the skin, without making a skin incision. This allows many of the procedures to be performed under local anesthesia without the need for extensive skin flaps and thus, more conservative operations.

In a subsequent trial from MCC, only those patients who had a positive SLN were subjected to a complete node dissection. Further micrometastatic disease was found in 22% of the patients who had a positive SLN and eventually had a complete node dissections, reinforcing the need for a complete node dissection in patients with a positive SLN.[39] In a series reported from MCC and M. D. Anderson, 423 patients with intermediate thickness melanoma underwent preoperative lymphoscintigraphy and intraoperative mapping with the Neoprobe. The SLN was identified and harvested. Of the 612 patients, 423 (69%) had negative SLN biopsies and were followed. With a mean follow-up of 18 months, nine patients (2.1%) have had recurrences in a regional basin that was mapped with a negative SLN. More detailed examination with serial sectioning and immunohistochemical staining of the block from the original SLN harvest found metastatic melanoma in seven of the nine patients, suggesting that the majority of recurrences were due to missed micrometastatic disease in the SLN by the pathologist.[40] The recurrences in a nodal basin that had a previous negative SLN biopsy were due to failures in the pathologic examination and not the surgical technique.

A National Cancer Institute-sponsored prospective randomized trial[41] currently is being performed that randomizes patients to receive either wide local excision of the primary melanoma site *versus* wide local excision and SLN biopsy of the regional lymphatic basins at risk for metastases. The end point of the study is whether this surgical strategy can extend the survival of the patient with melanoma. The study is different than the previous randomized trials addressing the efficacy of ELND because only a percentage of the patients with melanomas greater than 1 mm will receive a complete node dissection. Even if this trial is a negative trial, with the recent data on the efficacy of adjuvant IFN-α-2B in high-risk patients, most of whom had nodal metastases,[42] it may be necessary to perform a nodal staging procedure on all patients with melanomas greater than 1 mm. Lymphatic mapping and selective lymphadenectomy is the less morbid, less costly procedure, compared with ELND, in obtaining nodal staging.

It cannot be understated how important it is for the surgeon who performs intraoperative mapping to have adequate nuclear medicine and pathology support. Lymphatic mapping requires the close collaboration of nuclear medicine, surgery, and pathology to perform the procedure accurately. The preoperative lymphoscintigraphy (Fig. 3) is a road map for the surgeon and is used for four distinct reasons in planning the surgical procedure. These include:

1. Identifying all nodal basins at risk for metastatic disease;
2. Identifying any in-transit areas of nodal collections that can be tattooed by the nuclear medicine colleague for later harvesting; in-transit metastases occur in 5% of the melanoma population and may, by definition, be considered the SLN;
3. Identifying the location of the SLN in relation to the rest of the nodes in the basin; and
4. Estimating the number of SLN in the regional basin that will need to be harvested.

Intraoperatively, vital blue dye and radiocolloid mapping with the Neoprobe can identify the blue staining afferent lymphatic draining into the blue staining and hot SLN (Fig. 4). Then this SLN can be harvested, allowing the pathologist to make a detailed examination of the SLN

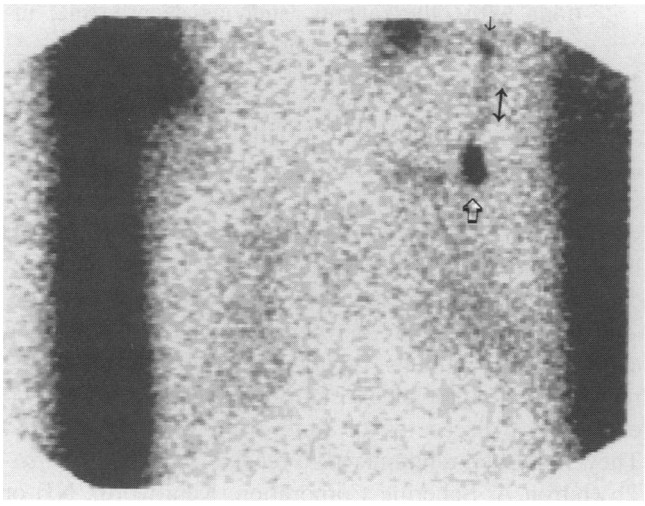

Figure 3. Preoperative lymphoscintigraphy in a patient with an intermediate-thickness melanoma on the left arm. The left arm is held above the head to maximize distance between the primary site and the regional basin. The body outline is masked behind the lymphoscintigraphy. Injection around the primary site and imaging at 10 minutes shows an afferent lymphatic (double-headed arrow) leading to an in-transit node (outside the classic regional basin, small solid arrow). This in-transit node is located in the upper medial arm. In addition, a sentinel lymph node (SLN) is identified in the left axilla (open arrow). Both nodes were harvested and both contained metastatic melanoma. After a complete node dissection, the SLN was the only site of disease in the axilla.

with multiple sections, immunohistochemical staining with melanoma-specific monoclonal antibodies, HMB-45 and S-100, and perhaps RT-PCR determination. This strategy will invariably increase the yield of positive dis-

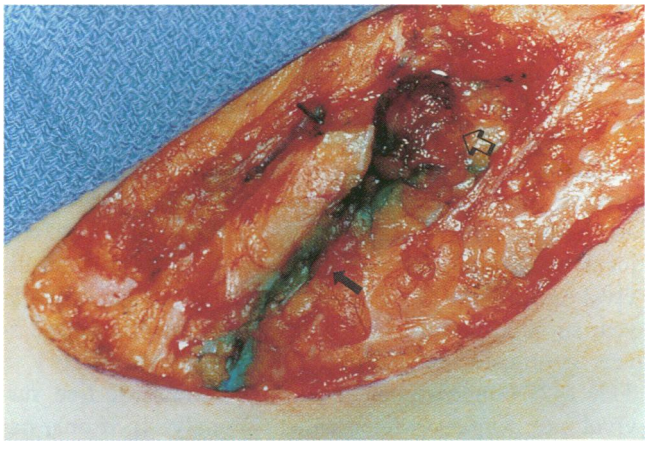

Figure 4. Intraoperative lymphatic mapping and sentinel lymph node (SLN) biopsy consists of injection of a vital blue dye and a radiocolloid around the primary melanoma site. Within minutes, the dye and tracer are taken up by the afferent lymphatics. An incision over the nodal basin will find a blue-staining afferent lymphatic (closed arrow) draining into a blue staining, hot SLN (open arrow). The SLN is harvested for histologic examination.

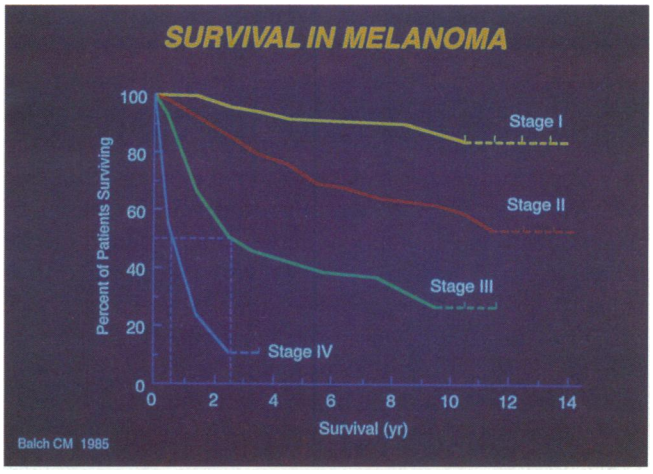

Figure 5. The American Joint Committee on Cancer staging system for melanoma separates patients into low and high risk for recurrence and death groups. Patients with stage III melanoma (regional nodal disease) have a greater than 50% chance of recurrence and death within 5 years of diagnosis of their melanoma. These patients are candidates for the adjuvant therapy trials.

sections and more accurately stage the patient with melanoma.

The question then becomes what constitutes the standard of surgical care for the patient with melanomas greater than 1 mm in thickness. With five reports in the literature that illustrate how the histology of the SLN is reflective of the histology of the rest of the nodes in the basin, if surgeons have adequate support from nuclear medicine and pathology services, there is no need to perform an ELND. In those communities and centers that do not have the collaboration in place, or in circumstances in which intraoperative mapping cannot be performed (such as when a previous wide local excision of the primary melanoma already has been performed) or when the results of the mapping are equivocal, the guidelines for ELND from the Intergroup Melanoma Surgical Trial should be used.

ADJUVANT INTERFERON-ALPHA-2B (INTRON A) THERAPY FOR THE TREATMENT OF HIGH-RISK STAGE IIB AND III DISEASE

Advances in melanoma treatment during the last decade have evolved primarily from more detailed knowledge about prognostic factors of primary and metastatic lesions. Within the larger group of melanoma patients who undergo potentially curative treatment by surgical resection and have no evidence of disease, subgroups can be identified who are at high risk for recurrence and for development of systemic metastases. Patients with melanomas of 1.51- to 3.99-mm depth have a variable and

intermediate risk of relapse, whereas patients with thick primary melanomas (>4 mm thick), in-transit lesions, and regional lymph node involvement are at particularly high risk: the 5-year relapse risk of these stage groupings generally exceeds 50%. The American Joint Committee on Cancer staging system correlates the stage of disease with prognosis (Fig. 5) and identifies subpopulations of patients with melanoma who have a high enough risk of recurrence and death at 5 and 10 years (deep stage II or stage III) that they are candidates for adjuvant trials. Once distant metastases develop, median survival is only 6 to 9 months. The investigation of adjuvant systemic treatment that can prevent melanoma recurrence has become a critical area of investigation, focused on the high-risk stage groups.

The rationale and general principles for adjuvant treatment of cancer are based on the premise that treatment, whether chemotherapy or immunotherapy, is more effective when the tumor cell population is small and host-immune and other resistance mechanisms still are in tact. To date, randomized trials using dacarbazine, nitrosourea, a variety of combination chemotherapy regimens, BCG, *Corynebacterium parvum,* transfer factor, and combinations of immunotherapy and chemotherapy have not demonstrated any advantage for treatment in an adjuvant fashion.

Adjuvant treatment for melanoma should be considered whenever possible within clinical research protocols that seek to improve on the results of current standard therapy in a systematic manner. Investigations during the last decade have focused on the IFNs in this category of high-risk melanoma, and four trials conducted in this interval recently have been published or presented in national and international forums. The Eastern Cooperative Oncology Group (ECOG) has completed a trial of adjuvant therapy with high-dose IFN-α-2b *versus* observation in stage IIB/stageIII (American Joint Committee on Cancer) patients, the results of which have served as a pivotal basis for Food and Drug Administration approval of IFN-α-2b (December 1995) for the adjuvant treatment of high-risk melanomas.

The rationale for this trial was based on several factors and included:

1. There was no curative therapy for relapse melanoma, particularly for systemic recurrences.
2. The IFNs have been shown to have antitumor, antiproliferative, and immunomodulatory effects *in vitro.*
3. Preliminary phase I and II clinical trial data suggested that IFNs had antitumor activity *in vivo.*
4. Subsequent trials showed that the α-IFNs, particularly IFN-α-2b, have activity against metastatic melanoma as single agent therapy and in combinations with other agents, such as dacarbazine, interleukin-2 or other biologic therapies.

The ECOG study E1684 was initiated to assess the effects of IFN-α-2b therapy as adjuvant therapy for high risk melanoma patients with no evidence of disease after surgical therapy. The end points of the study were overall survival and relapse-free survival. In addition, quality of life was measured using the Q-twist methodology, and a cost analysis was initiated. The trial was begun in 1985, stratifying patients into relevant prognostic factors known at the time. All patients had nodal staging, which included regional node lymphadenectomy to render them with no evidence of disease at the time of entry into the study. They were then randomized to receive 1 year of IFN-α-2b (Intron A, Schering Corporation, Kenilworth, NJ) or observation. The treatment protocol adopted for this early trial is notable for its use of an initial month of daily intravenous therapy at 20 MU/M^2 per day $\times$ 5 $\times$ 4 weeks. This 1-month period of induction therapy was designed to deliver peak levels of circulating IFN-α unattainable by other routes, and was followed by 11 months of subcutaneous therapy at 10 MU/M^2 three times a week, designed to sustain maximal tolerable levels of IFN in an outpatient/home therapy setting. Phase II studies had shown that this IFN-α-2b dosing regimen was near maximum tolerable dose and that the intravenous dosing demonstrated increase tolerability relative to other routes of administration. Baseline demographic and clinical characteristics were distributed similarly between the two arms of the study. Entry into the study was stratified with respect to clinical stage and pathologic stage. A requirement of the study was that patients should initiate adjuvant treatment within 56 days of surgery. Eighty-nine percent of the enrolled patients had node involvement (stage III disease). The study was closed in May 1993, by which time the medians for overall and disease-free survival had been met and the median follow-up was 6.9 years. An analysis of 280 of 287 patients for whom full data were available has demonstrated a significant prolongation of median time to relapse (1.7 *vs.* 0.98 years, $p = 0.005$) and overall survival (3.8 *vs.* 2.8 years, $p = 0.047$). Treatment was associated with an increase in the 5-year survival rate from 36% to 47% (24% improvement in the 5-year survival rate, Fig. 6), and with an increase in the 5-year continuous relapse-free survival rate from 26% to 37% (42% improvement in the 5-year disease-free survival rate; Kaplan–Meier statistical analysis). If only patients with nodal metastases are considered, the p value for disease-free survival decreases to 0.0006 and the p value for survival decreases to 0.006. The multivariate regression analysis of overall survival showed that stage of disease (stage III worse than stage II, $p = 0.002$), presence of an ulcerated primary ($p = 0.013$), patient age

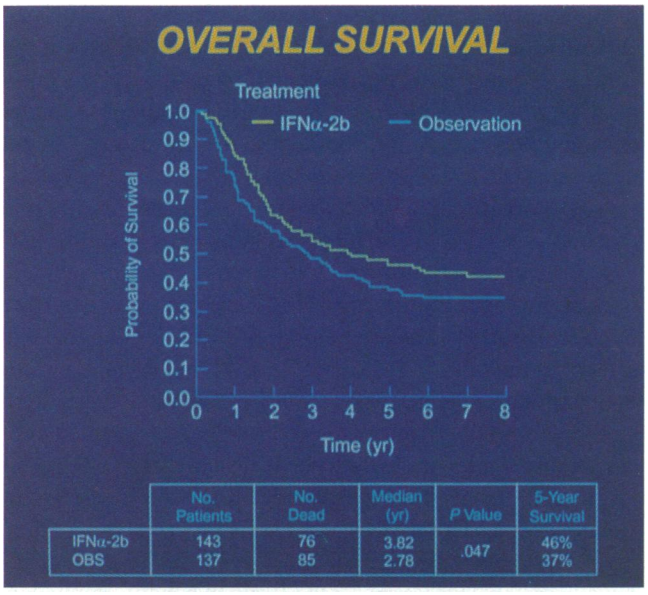

Figure 6. The overall survival curve for patients with "high risk for recurrence" melanoma who were randomized to receive 1 year of adjuvant interferon-alpha–2b (IFN-α–2b). Most of the separation of the curves occurred during the first and only year of therapy, but this separation was sustained with mature follow-up. There was a significant difference in survival in favor of the patients treated with IFN-α–2b ($p = 0.047$).

older than 50 years ($p = 0.032$), and treatment with IFN-α–2b ($p = 0.009$) significantly influence overall survival. These data have suggested the possibility of a curative impact of therapy with IFN-α–2b as given in this trial, and it would be difficult to not offer this therapy to patients with this magnitude of a survival difference, especially those with stage III disease.[42]

Interferon α-2b toxicities reported in the trial were significant but similar to other trials using this high dose. The safety profile was not unexpected, and there was no indication of different adverse responses between the induction and maintenance phase, nor was there any sign of cumulative toxicity from IFN-α–2b. Toxicity of this therapy is significant, with nearly ubiquitous flu-like symptoms of moderate to severe degrees and the need for dose delay or attenuation in more than one third of patients during therapy. The completion of 1 year of therapy was feasible in the majority (74%) of patients who did not experience progression, and proper attention to hematologic and hepatic function has been sufficient to avoid lethal hepatic toxicities observed early during the application of this intensive protocol. Adverse events, such as fever, myalgia, and nausea and vomiting, decreased during maintenance, and this was representative of some tolerance development with the therapy. Three treatment patients reported suicidal ideation, but no one attempted suicide. The frequency and pattern of side effects were not related to age or gender of the patient, and the age range was 18 to 82 years. An analysis of the quality of life showed that the benefits of increased time without symptoms or toxicities, and the improvement in overall survival strongly outweighed the risk of toxicities associated with IFN-α–2b.[43,44]

The ECOG has led an intergroup confirmatory sequel study (E1690/SWOG9111/CALGB9190) that completed accrual in mid 1995 and will require up to several years of maturity for evaluation. This protocol was designed to corroborate E1684, and to test the influence of a lower dose of IFN-α–2b, 3 MU/day (flat dose) given subcutaneously three times a week for 2 years. It closed in 1995 with accrual of 642 patients.

In addressing the cost/benefit issues with this trial, one uses the federally supported renal dialysis program as the benchmark for an acceptable cost that could be supported by our healthcare system. In this program, which is funded by Medicare and other insurance companies, $50,000 is spent for 1 year of life saved. The cost for 1 year of IFN-α–2b on this protocol was $26,000, which in the trial extended the survival of the treatment arm 1 year. Thus, the costs of the survival benefit certainly is in the range that has in the past been supported by our healthcare system. If adjuvant IFN-α treatment truly is preventing stage IV disease, cost savings are even more substantial, because on average it costs $100,000 at MCC in an attempt to treat stage IV melanoma. In addition, none of the patients are cured of stage IV disease. A more thorough cost analysis study is being prepared at this time.

In an accompanying editorial on this trial, Balch and Buzaid[44] questioned what the role of adjuvant IFN-α would be in a patient at high risk for recurrence whose tumor is greater than 4 mm thick and whose lymph node examination is clinically negative, without doing a node dissection. Although excluded from the ECOG eligibility criteria, these patients have a greater than 60% chance of harboring microscopic nodal metastases and therefore would benefit from adjuvant high-dose IFN therapy. Knowing the pathologic status of the regional nodes in patients with thick melanomas provides important prognostic information because patients with thick melanomas who have negative nodes have a significantly better prognosis than those patients with positive nodes (58–71% *vs.* 32–42%).[45] Because the available data from the ECOG trial applied only to patients whose pathologic status of the lymph nodes was known, and because there was no demonstrable benefit for the small subgroup of patients with thick, lymph node-negative tumors, these authors recommended that patients with melanomas greater than 4 mm have a nodal staging procedure, preferably intraoperative lymphatic mapping and SLN biopsy because this is the least morbid approach. Patients with local recurrences, satellite lesions, or in-transit metastases

also could be considered for adjuvant IFN-α–2b. Important questions that remain to be answered are whether the high-dose intravenous induction is necessary, whether a more prolonged IFN administration would enhance the benefit, and whether early IFN administration (after the resection of microscopic nodal metastases with ELNDs or SLN biopsies) is more effective than late IFN administration (after the resection of grossly positive nodes, therapeutic lymph node dissection).

Only a small subset of patients who receive high-dose IFN therapy actually benefit from it. A promising marker, serum tyrosinase (key enzyme in melanin biosynthesis whose gene is expressed actively only in melanocytes, melanoma cells, and Schwann cells) may be able to identify which patients actually are benefiting from the therapy. Because melanocytes and melanoma cells usually do not circulate in the blood, the detection of tyrosinase messenger RNA by RT-PCR in blood is considered an indication of the presence of melanoma cells.[46] Patients who have positive pretherapy RT-PCR results that subsequently become negative may be those who actually benefit from the therapy.

Other cooperative groups have studied the role of other formulations and other doses of IFN-α for the adjuvant treatment of patients at high risk for recurrence melanoma. The Northcentral Cancer Treatment Group (NCCTG 83-7052) evaluated the adjuvant role of IFN-α–2a given at 20 MU/M^2 three times a week for 3 months *versus* observation in patients with greater than 1.69 mm Breslow Depth T3 + T4, and N1 patients of American Joint Committee on Cancer stages IIA/B, and III. An analysis of 260 evaluable patients who entered this trial demonstrates no significant prolongation of survival, or of relapse-free interval overall. Subset evaluation of patients with stage II *versus* those with stage III participating in this trial reveal an impact in the latter, by Cox analysis.[47]

The WHO Melanoma Program Trial #16 has evaluated the efficacy of a lower dosage of IFN-α–2a, given 3 MU subcutaneosly three times a week for 3 years *versus* observation. Of 444 patients who entered this trial, a majority exhibited extracapsular extranodal involvement, a pathologic variable that made patients ineligible for the trials of the U.S. Cooperative groups previously noted. An analysis of this trial at intervals up to 39 months of median follow-up have suggested the absence of a significant impact on either relapse-free or overall survival.[48]

The evaluation of newer and more effective biologic agents in the adjuvant setting has been pursued in multiple trials. The Southwest Oncology Group (SWOG) 86-42 is a trial of IFN-γ administered at dosages projected to be the optimal immunomodulatory dosage (0.2 mg/day subcutaneously every other day for 1 year). Interferon-γ is among the most potent immunomodulators yet tested, and one for which initial hopes for adjuvant and metastatic disease applications were elevated even beyond IFN-α–2. Unfortunately, the therapeutic activity of IFN-γ in the advanced disease setting has been negligible. The recently published the Southwest Oncology Group 86-42 trial demonstrates the lack of therapeutic benefit for this agent at the dosage tested, in either intermediate-risk stage II- or stage III-resected melanoma. Although it is notable that the initial report of this trial suggested potential adverse impact of treatment that has not been confirmed, the unequivocal failure of IFN-γ to improve relapse-free or overall survival in the adjuvant setting argues that the effects of the dose/route/schedule of IFN-γ and the monocyte and natural killer cell activation that have been associated with IFN-γ administered as in this trial are not sufficient to alter the outcome of this disease.[49]

Thus, the analyses of E1684, NCCTG 83-7052, Southwest Oncology Group 86-42 and WHO #16, taken together, argue that higher dosages of IFN-α–2b, delivered intravenously, may be necessary for the survival benefit. Equivalent dosages of IFN-α–2a administered by the intramuscular route for shorter periods have not been effective, and lower dosages administered by the subcutaneous route for longer periods have been ineffective to date. The approval of IFN-α–2b for adjuvant therapy within 2 months of surgery of high-risk, node-positive, and deep primary melanoma is the first adjuvant therapy approved for melanoma, and the first new agent approved for therapy of this disease in any stage or setting, since dacarbazine.

Which of the pleiotropic effects of IFN-α–2b that account for its therapeutic benefit in melanoma have yet to be proven. *In vivo* activities that may be important range from direct antiproliferative and cytotoxic actions, to immunopotentiation of T or B cell host responses, dendritic cell antigen presentation, angiogenesis factor inhibition, and tumor cell histocompatibility and tumor-restricted antigen expression. These are being evaluated prospectively in the context of a recently completed intergroup trial (ECOG 1690/SWOG 91-11, CALGB 91-90).[50]

SUMMARY

In the last 2 years, there has been a number of developments in the surgical and medical care of the melanoma patient that promise to change the standards of care. Stimulated by new molecular biology techniques, more sensitive assays for occult metastatic disease promise to identify subpopulations so that adjuvant therapy can be applied in a selective fashion. Nodal staging becomes more important to obtain on all patients with melanoma greater than 1 mm, and the easiest, most cost-effective way to accomplish this is with preoperative lymphoscintigraphy, intraoperative mapping, and sentinel node biopsy. This approach allows the pathologists to concentrate their examination on I or II nodes, and apply either serial sec-

tioning and immunohistochemical staining or RT-PCR assays to identify the occult metastases. If clinical correlation is proven with the new RT-PCR assays, combined with the effective adjuvant IFN-α-2b therapy for high-risk melanoma, especially in those with documented nodal metastases, more than just stage shifting occurs.[51] In this way, melanoma care becomes more rational, conservative, and economical.

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